Figure 1 A

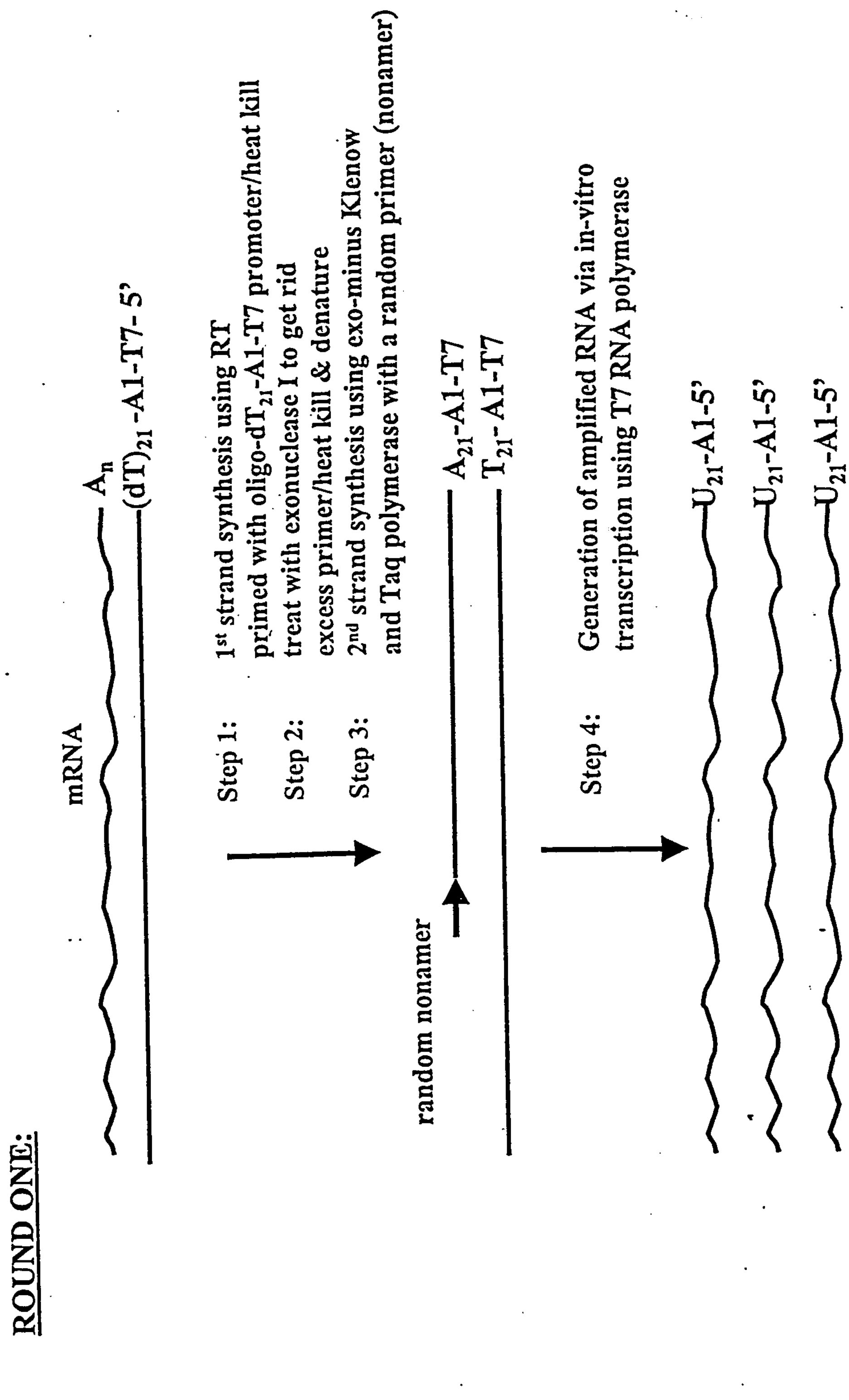

ROUND ONE:
mRNA
A n
(dT) 21 -A1-T7- 5'
Step 1: 1st strand synthesis using RT primed with oligo-dT 21 -A1-T7 promoter/heat kill
Step 2: treat with exonuclease I to get rid excess primer/heat kill & denature
Step 3: 2nd strand synthesis using exo-minus Klenow and Taq polymerase with a random primer (nonamer)
random nonamer
A 21 -A1-T7
T 21 - A1-T7
Step 4: Generation of amplified RNA via in-vitro transcription using T7 RNA polymerase
U 21 -A1-5'
U 21 -A1-5'
U 21 -A1-5'

Figure 1B

ROUND TWO:

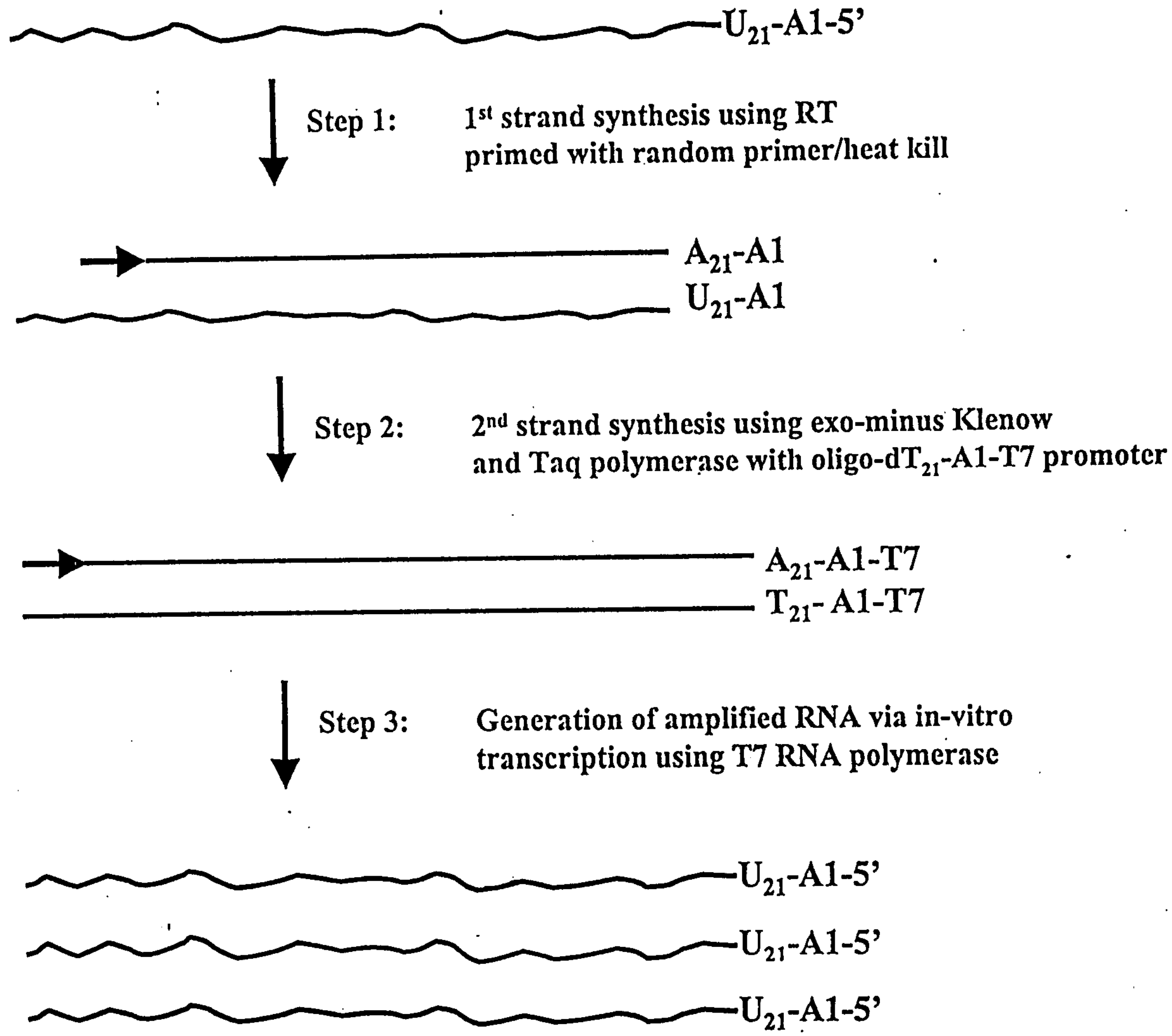


Figure 1C

ROUND TWO *MODIFIED*:

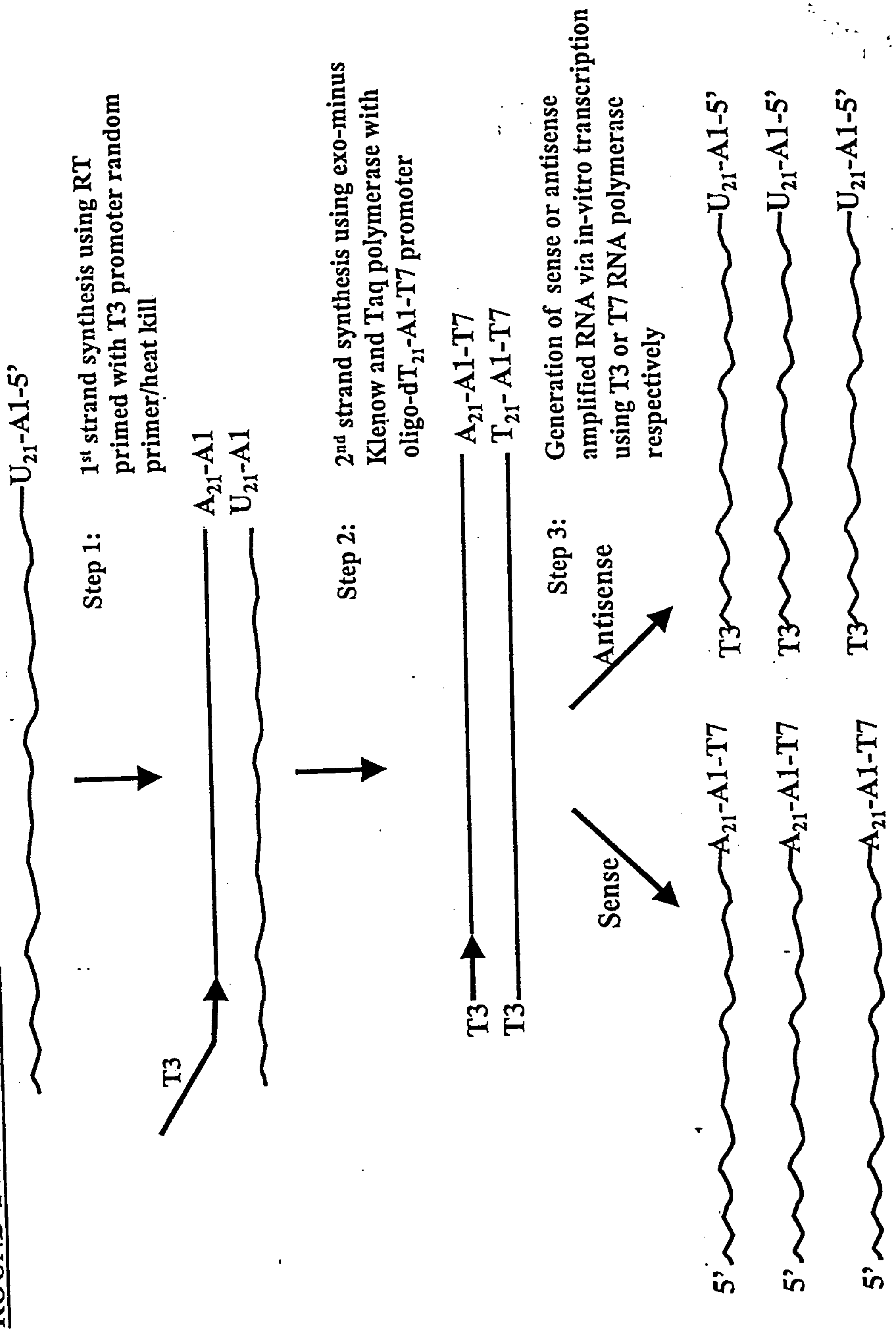
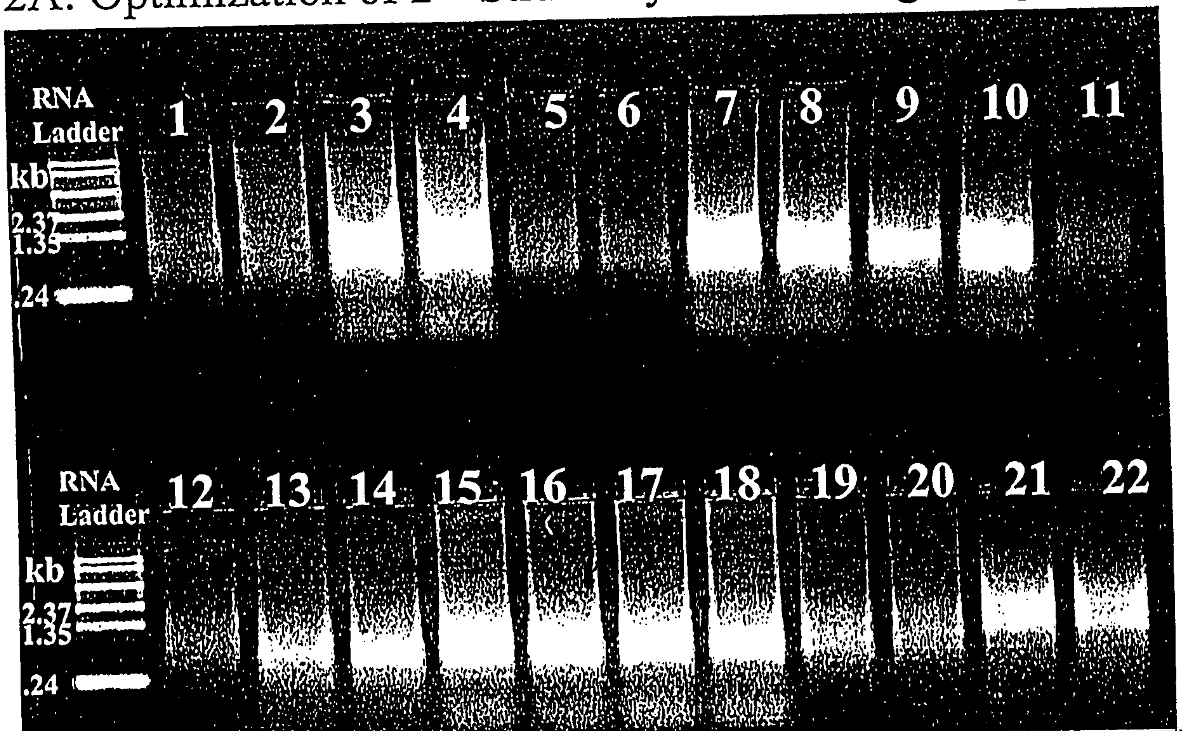


Figure 2A: Optimization of 2nd Strand Synthesis using Exogenous Primers



1	no RNaseH, no Primer, Exo-Klenow and Taq
2	no RNaseH, no Primer, Exo-Klenow and Taq
3	no RNaseH, with Primer, Exo-Klenow and Taq
4	no RNaseH, with Primer, Exo-Klenow and Taq
5	with RNaseH, no Primer, Exo-Klenow and Taq
6	with RNaseH, no Primer, Exo-Klenow and Taq
7	with RNaseH, with Primer, Exo-Klenow and Taq
8	with RNaseH, with Primer, Exo-Klenow and Taq
9	with RNaseH, with Primer, Exo-Klenow alone
10	with RNaseH, with Primer, Exo-Klenow alone
11	with RNaseH, with Primer, Taq alone

12	with RNaseH, with Primer, Taq alone
13	with RNaseH, with Primer, Sequenase alone
14	with RNaseH, with Primer, Sequenase alone
15	with RNaseH, with Primer, regular Klenow alone
16	with RNaseH, with Primer, regular Klenow alone
17	with RNaseH, with Primer, regular Klenow and Taq
18	with RNaseH, with Primer, regular Klenow and Taq
19	with RNaseH, with Primer, Reverse Transcriptase alone
20	with RNaseH, with Primer, Reverse Transcriptase alone
21	endogenous priming with DNA Pol1,Ligase and RNaseH
22	endogenous priming with DNA Pol1,Ligase and RNaseH

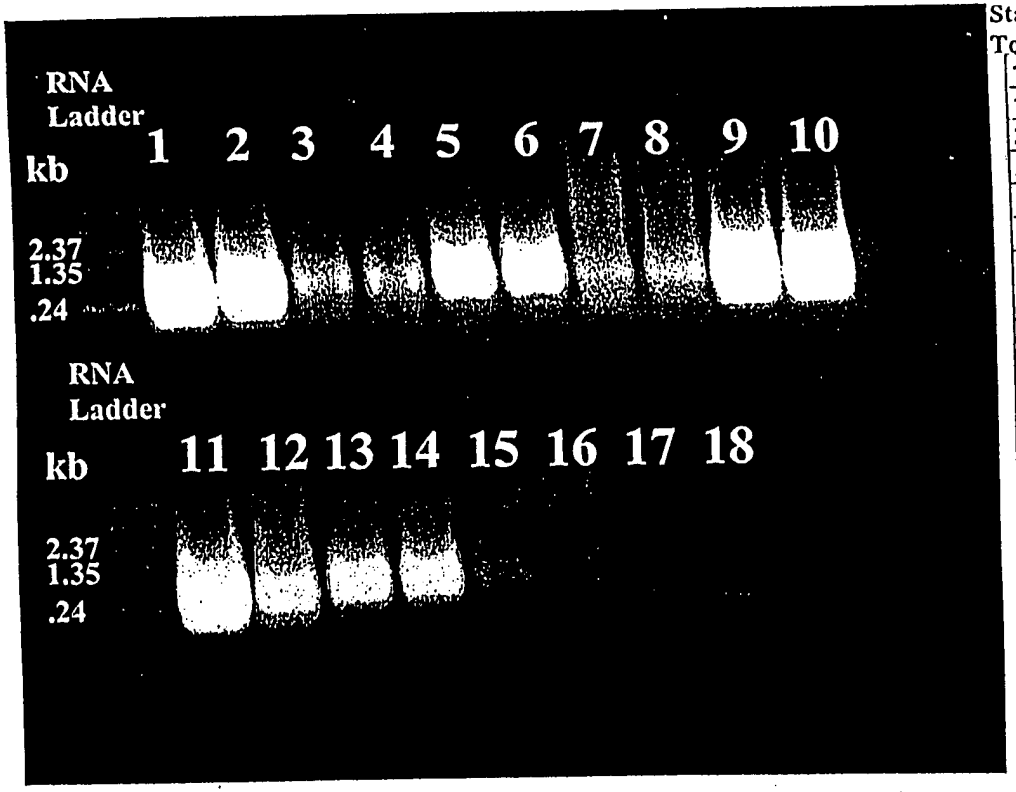
Figure 2B: Yields From Exogenous Priming of 2nd Strand Synthesis Using Different Enzymes

SAMPLES	Condition Tested	ug of amplified RNA
1	no RNaseH, no Primer, Exo-Klenow and Taq	3.6
2		3.4
3	no RNaseH, with Primer, Exo-Klenow and Taq	15.5
4		19.2
5	with RNaseH, no Primer, Exo-Klenow and Taq	3.4
6		3.0
7	with RNaseH, with Primer, Exo-Klenow and Taq	16.9
8		17.5
9	with RNaseH, with Primer, Exo-Klenow alone	18.7
10		16.8
11	with RNaseH, with Primer, Taq alone	2.8
12		3.6
13	with RNaseH, with Primer, Sequenase alone	9.0
14		10.4
15	with RNaseH, with Primer, regular Klenow alone	16.0
16		15.2
17	with RNaseH, with Primer, regular Klenow and Taq	13.7
18		15.2
19	with RNaseH, with Primer, Reverse Transcriptase alone	7.2
20		6.5
21 Eberwine1	endogenous priming method with DNA Pol1,Ligase and RNaseH	10.2
22 Eberwine2		11.7

Figure 2C: Comparison of Yields and Fold Amplifcation				
SAMPLES	Condition Tested	ave (ug)	fold diff vs GH	est. fold amp*
1	no RNaseH, no Primer, Exo-Klenow and Taq	3.5	0.3	174
2				
3	no RNaseH, with Primer, Exo-Klenow and Taq	17.3	1.6	865
4				
5	with RNaseH, no Primer, Exo-Klenow and Taq	3.2	0.3	159
6				
7	with RNaseH, with Primer, Exo-Klenow and Taq	17.2	1.6	862
8				
9	with RNaseH, with Primer, Exo-Klenow alone	17.7	1.6	887
10				
11	with RNaseH, with Primer, Taq alone	3.2	0.3	161
12				
13	with RNaseH, with Primer, Sequenase alone	9.7	0.9	486
14				
15	with RNaseH, with Primer, regular Klenow alone	15.6	1.4	778
16				
17	with RNaseH, with Primer, regular Klenow and Taq	14.4	1.3	721
18				
19	with RNaseH, with Primer, Reverse Transcriptase alone	6.8	0.6	342
20				
21 Eberwine1	endogenous priming method with DNA Pol1,Ligase and RNaseH	11.0	1.0	548
22 Eberwine2				

*fold-amplification calculated as follows: (final μg yield)/(0.020 μg) where 0.020 μg is an estimate based on the assumption that 2% of 1 μg of total RNA (the amount of starting material) is poly(A) RNA

Figure 3A:



Starting Total RNA		
10 ng	1	Eberwine Method
10 ng	2	Eberwine Method
2 ng	3	Eberwine Method
2 ng	4	Eberwine Method
10 ng	5	Exo-klenow/ Taq with T7A1
10 ng	6	Exo-klenow/ Taq with T7A1
2 ng	7	Exo-klenow/ Taq with T7A1
2 ng	8	Exo-klenow/ Taq with T7A1
10 ng	9	Exo-klenow/ Taq with T7A1dT
10 ng	10	Exo-klenow/ Taq with T7A1dT
2 ng	11	Exo-klenow/ Taq with T7A1dT
2 ng	12	Exo-klenow/ Taq with T7A1dT
10 ng	13	Regular klenow/Taq with T7A1
10 ng	14	Regular klenow/Taq with T7A1
2 ng	15	Regular klenow/Taq with T7A1
2 ng	16	Regular klenow/Taq with T7A1
0 ng	17	negative control--no RNA
0 ng	18	negative control--no RNA

Figure 3B:

Total RNA			conc (ng/ml)	yield
10 ng	1	Eberwine method	1860	101.0
10 ng	2	Eberwine method	1800	97.4
2 ng	3	Eberwine method	448	26.9
2 ng	4	Eberwine method	439	26.3
10 ng	5	exo-klenow + taq with t7a1	946	46.2
10 ng	6	exo-klenow + taq with t7a1	945	46.1
2 ng	7	exo-klenow + taq with t7a1	518	20.5
2 ng	8	exo-klenow + taq with t7a1	464	17.2
10 ng	9	exo-klenow + taq with t7a1dt	1700	91.4
10 ng	10	exo-klenow + taq with t7a1dt	1825	98.9
2 ng	11	exo-klenow + taq with t7a1dt	2400	144.0
2 ng	12	exo-klenow + taq with t7a1dt	648	38.9
10 ng	13	regular klenow + taq with t7a1	780	36.2
10 ng	14	regular klenow + taq with t7a1	808	37.9
2 ng	15	regular klenow + taq with t7a1	313	8.2
2 ng	16	regular klenow + taq with t7a1	298	7.3